A Synopsis

on

DEVELOPMENT OF EMULGEL-BASED TOPICAL DOSAGE FORMS OF ANTI-PSORIATIC PHYTOACTIVE OF BETULA UTILIS: OPTIMIZATION, CHARACTERIZATION, AND EVALUATION

SYNOPSIS OF THE THESIS

Submitted by

Anjali Chaudhary

Registration No. AU21PYPhD0075

Under supervision of

DR. DEV PRAKASH DAHIYA

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF PHARMACY

FACULTY OF PHARMACY

ABHILASHI UNIVERSITY

CHAIL-CHOWK, MANDI (H.P.) – 175028

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DECLARATION

I hereby declare that the dissertation synopsis entitled "Development of Emulgel-based Topical Dosage Forms of Anti-psoriatic Phytoactive of *Betula utilis*: Optimization, Characterization, and Evaluation" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

Anjali

Place: Chail-Chowk

Date: 28-11-2023

Signature of the Scholar

Anjali Chaudhary

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Date: 28-11-2023

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1. Introduction

Psoriasis has emerged as a global epidemic and the leading cause of mental distress that promotes discrimination in employment and social isolation (1, 2). The global incidence of psoriasis is about 2%, while its incidence in developed countries is higher, i.e., about 4.6%. About two out of the three people with psoriasis live with its mild (when less than three per cent of the skin is affected) form (3). About 75% of the psoriatic patients are from the age group of 20-40 years; however, 25% appeared before the age of 20 years.

It is an immune-mediated chronic inflammatory disorder of the skin characterized by the hyper-proliferation of keratinocytes and massive infiltration of leukocytes (4). Silvery scales and plaques develop on the skin due to epidermal proliferation, altered metabolic activities, and the invasion of immune cells in the dermis and epidermis (5). It rarely covers the whole surface of the skin. Severe forms of this disease can be lethal because severe inflammation and breakage of the surface disrupt the body's ability to regulate homeostasis and barrier functions.

Source: https://www.futura-sciences.com/sante/questions-reponses/corps-humain-psoriasis-symptomes-traitements-7293/

The main histopathological features of psoriasis are, hyper-proliferation of keratinocytes (acanthosis – thickening of the epidermis), epidermal hyperplasia and elongated dermal

papillae. Another histological feature is the dilatation and elongation of capillaries almost to the surface of the skin. Third change is infiltration of neutrophils, lymphocytes, macrophages and dendritic antigen-presenting cells in dermis and epidermis (6). This disease is diagnosed based on the presence of typical skin lesions. No specific blood test or diagnostic procedure available till date that may confirm the occurrence of psoriasis. In some cases, a skin biopsy or scraps are needed to confirm the diagnosis.

Source: https://hellodoctor.com.ph/skin-health/psoriasis/gamot-sa-psoriasis/, https://www.verywellhealth.com/how-psoriasis-is-diagnosed-2788316

Unfortunately, no cure available till date, that may completely cure psoriasis. Its treatment schedule highly depends on alleviating psoriasis specific symptoms. Several anti-psoriatic drugs are available in the market which reduce the symptoms associated with psoriasis. Based

on route of administration, treatment options are classified in the following types such as biologics/parenteral, phototherapy, oral treatments and topicals.

1. *Biologics: TNF-α inhibitors* (certolizumab pegol, etanercept, adalimumab, infliximab and golimumab), *IL-12 and IL-23 inhibitor* (ustekinumab), *IL-17 inhibitor* (secukinumab and ixekizumab) and *T cell inhibitor* (abatacept) are used as biological to treat psoriasis.
2. *Systemics:* Systemic medications are prescribed for individuals who are moderate to severely affected by psoriasis. Systemic medications are prescribed for the unconscious patients or in the patients in whom topical medications cannot be administered. These are taken in the form of pills and injections. Some of the examples of this category are acitretin (Soriatane), Cyclosporine and Methotrexate.
3. *Phototherapy:* In this therapy, psoriasis skin is exposed to UV light intermittently under the advice of a medical practitioner. This is done in a clinic equipped with such facility under much monitored conditions. Lights which are used in this therapy are ultraviolet, sunlight and laser.
4. *Oral treatments:* These drugs are stable at GIT conditions and hence these are developed as oral treatments. These drugs are meant to target receptors situated inside immune cells. These adjust the complex inflammatory processes within the cell and help correct the overactive immune responses. One of the wide known examples is Otezla that treats psoriasis by inhibiting phosphodiesterase 4 activity within inflammatory cell.
5. *Topicals:* Psoriasis like inflammations have been treated topically since many years ago with the help of coal tar derivatives and salicylic acid (7). Folic acid antagonists (8, 9), cyclosporine (10), ionization radiation therapy, combinations of ointments and polyethylene films, sunlight and ultraviolet bath (11-14) are proved to be the other form of psoriasis treatments. These are the first line of treatment against psoriasis that slow down or normalize higher proliferation of keratinocytes. Out of effective topical options, some of them are procured over the counter and most of them are prescribed by the dermatologists. Corticosteroids are the most preferred topicals. They act as anti-inflammatory agents and reduce swelling and redness. Additionally, anthralin, vitamin A, salicylic acid and coal tar are also used as topical treatments. Topical agents are also used as adjuvant with either ultraviolet light or systemic medications against moderate-to-severe psoriasis. Emollients are other useful adjuncts for

topical treatment of psoriasis. Salicylic acid is used in combination with other topical therapies such as topical corticosteroids (TCS) and calcineurin inhibitors for the treatment of thick limited plaques to increase the absorption of the latter into the psoriatic plaques. Low- to mid-potent TCS are used in facial/flexural psoriasis and high potency TCS are used in palmoplantar or thick psoriasis lesions. Non-corticosteroid treatment can facilitate the avoidance of long-term TCS use. Tacrolimus and pimecrolimus can be used for the treatment of facial and intertriginous psoriasis. Tazarotene is indicated for stable plaque psoriasis usually in combination with other therapies such as TCS. Vitamin D analogues alone or in combination with TCS are useful in stable plaques over limbs and palmoplantar psoriasis. Topical therapies for scalp psoriasis include TCS, Vitamin D analogues, salicylic acid, coal tar and anthralin in various formulations such as solutions, foams and shampoos. TCS, vitamin D analogues and tazarotene can be used in the treatment of nail psoriasis (15).

1.1. Problems associated with the current anti-psoriatic treatments

Dithranol is effective but it stains skin and cloths of the psoriatic patients and causes irritation over the skin. Corticosteroids are effective and convenient however associated with risk of cutaneous atrophy and remission of psoriasis after termination of therapy. Vitamin d analogues like calcipotriol and tacalcitol are effective but calcipotriol may cause irritation over the skin and tacalcitol is associated with hypercalcaemia. Efficiency of retinoids viz. tazarotene is limited and it causes irritation over the skin. Emollients are used to maintain the hydration of the skin. Combination of methotraxate and cyclosporine with corticosteroids (mometasone, dexamethasone etc.) are other treatment options which have been extensively investigated and currently being practiced (10). However, many of them are found to be associated with serious side effects viz. cutaneous atrophy, organ toxicity (hepatotoxicity, nephrotoxicity, teratogenicity), carcinogenicity and broadband immunosuppression, inefficacy, inconvenience to the patients which are limiting their long-term use. Short-term treatment options lead to remission of the disease after finishing the treatment (9, 16-18). Surveys carried out on psoriatic patients show high degree of disappointment due to the hopelessness associated with the available products in the market. Therefore, the investigations on new alternative treatments for psoriasis with fewer side effects were desirable.

1.2. Complementary and alternative medicine

Several plant-based drugs can meet these requirements and have been observed as promising agents for psoriasis treatment. Their acceptance among patients is very high as they are supposed to be safer than that of other conventional therapeutics. Broad structural diversity and multidirectional mechanisms of action make them cost effective and effective treatment option. Using traditional system or alternative system of medicine against psoriasis might solve the problems associated with the current medication systems up to the considerable extent. As per the available literatures, plant-based leads have been studied well against systemic as well as topical inflammations. For instance, natural triterpenoid have been used alternatively targeting NF-kB which was able to alleviate the psoriasis specific symptoms in mouse model (19). When curcumin was administered topically, it strongly alleviated IMQ induced psoriasis. It restored the epidermal hyperplasia and mRNA levels of IL-17A, IL-17F, IL-22, IL-1β, IL-6 and TNF-α (20).

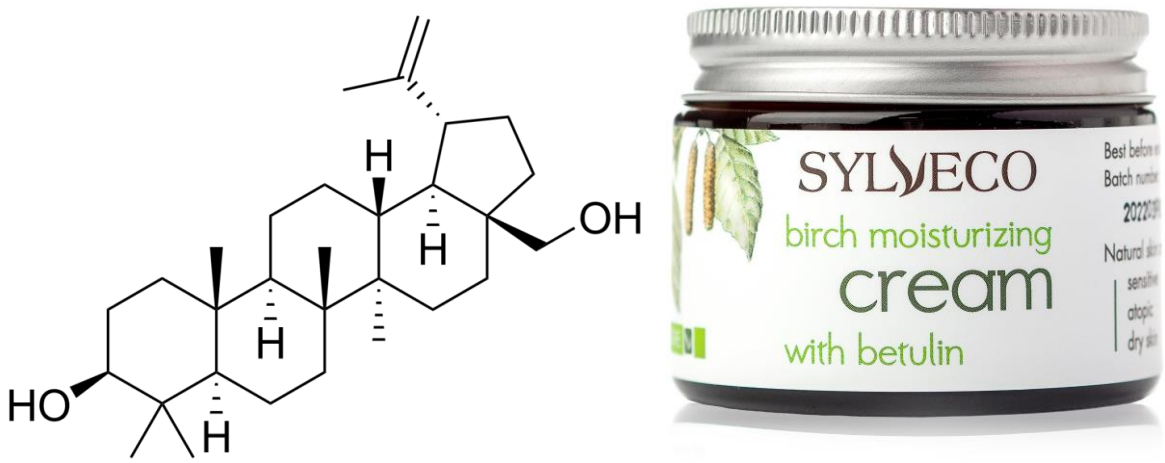
1.3. Probable treatment options

There are some plant-based drugs which reduce the level of psoriasis like symptoms. First in the list is bergamot polyphenolic fraction which inhibits spinal oxidative stress in the attenuation of morphine induced pain at 5-50 mg/kg dose. This was hypothesized due to the alleviation of superoxide and malondialdehyde and glutamate levels (21). Additionally, bergamot essential oil liposomes were developed to improve anti-cancerous effect on human neuroblastoma cells by improving water solubility of the phytocomponents (22). Wei et al. have evaluated antioxidant/lipoxygenase inhibitory activities of bergamot oil along with 24 essential oils in 2010 (23).

Eucalyptol (1,8-cineole), the main constituent of eucalyptus oil and known for anti-inflammatory effect, interacts with the transient receptor potential cation channels of various targets. Caceres et al. 2017 have carried out an investigation citing TRPM8 channels as the essential target for the anti-inflammatory effects of eucalyptol in mice (24). Anti-inflammatory and analgesic properties of *Eucalyptus citriodora* essential oil (citronellal 83.50 %) were also assessed in 2013 on Wistar rats. It showed very good analgesic and antipyretic effect i.e. comparable to 50 mg/kg of acetylsalicylate of lysine (a soluble salt of aspirin) (25).

Natural bioactives, Biswasroy et al. 2023 has summarized the probable role of various natural bioactive agents such as *Psoralea corylifolia*, *Nigella sativa*, *Curcuma longa*, *Capsicum annum*, *Smilax china*, *Woodfordia fructicosa*, and many more in treating psoriasis owing to their anti-inflammatory and anti-proliferative potentials. However, the altered physicochemical properties of these bioactives like inadequate drug absorption, lack of specificity in drug release, and unintended skin reactions were highlighted as the major restriction behind their ill use against the said disease. Although overcoming these drug delivery challenges and enhance the anti-psoriatic activity could be easily achieved by utilizing various lipidic system approaches. Liposome, ethosome, liposphere, and others have been reported to improve the physicochemical properties of such kind of bioactives and have been proved efficient in improvising solubility, skin penetration, bioavailability, bioactivity, and minimize undesirable effects (26).

Betulin, In another investigation (2022) of her report the anti-psoriatic potential of Betulin that is the major component of *Betula utilis.* The *in silico* computational analysis portrayed great utility of this component against pro-inflammatory cytokine specifically towards the overexpressed Glut-1 receptor. Its anti-psoriatic effect was validated using imiquimod (IMQ 5%) induced psoriasis-like skin inflammation model in small animal. Based on the psoriasis area and severity index (PASI) scores, macroscopic and behavioral evaluation, splenomegaly, cytokine levels and histological changes, its 180 mg/kg dose showed tremendous utility in comparison to the standard Betamethasone di-propionate ointment reflecting reduced expression of proinflammatory cytokine as well as restoring histological changes (27).



Source: https://lyko.com/de/sylveco/sylveco-birch-cream-with-betulin-50-ml

1.4. Challenges specific to psoriasis and the development of anti-psoriatic topical products

Epidermis of psoriatic skin can be thickened and/or thinned on account of which one may get higher variability in drug permeation and systemic absorption. These heterogeneities in the skin morphology offer various challenges for the formulation scientists. Most of the patients with psoriasis feel that the current therapies are not sufficiently efficacious and aggressive therefore, development of novel therapies with quick response in once-a-day topical administration is very much required. Most of the therapies are used in combination today, therefore to achieve optimum response with minimum or no side-effects. Appropriate cosmetic elegance, ease of application on normal as well as hair exposed skin area, no or minimal staining on clothing and bedding, quick absorption through skin and less greasiness may increase patient acceptance. Competitive cost of the product is also an important parameter to influence physician's and patient's interests.

1.5. Dosage form designing

To designed a dosage form for topical application, one must deal with various safety aspects and physicochemical characteristics of essential oils and their molecules. Most of the essential oils and their components are volatile in nature and therefore, need special attention while developing their dosage forms. Terpenes, their alcohols, and acetates are colourless to pale yellow liquids with low vapor pressure, acidic nature and low solubility in water. Despite their liquid state, slightly soluble nature, acidic pH and low vapor pressure, these molecules are reported to show mild irritation over the skin (in pure form), volatile and higher penetration ability through skin (28). Heterogeneity in the skin morphology of the psoriatic patients might increase the variability in the permeation of these kind of drugs and hence it was another point of concern while designing their semi solid anti-psoriatic formulation.

1.6. Novel drug delivery systems

Novel Drug Delivery Systems can be very useful to overcome shortcomings associated with the conventional drug delivery systems. In a search to find a way around, variety of nanocarrier based approaches, such as dendrimers (29), polymeric nanoparticles (30), liposomes (31-33) and nanoemulsions (34, 35) have been reported. These are developed to increase the absorption and distribution of the drug molecules to the different skin layers in a controlled manner. They have been used in an array of applications for instances, providing carrier for volatile constituents, thick texture to the molecules of liquid state, maintaining pH

similar to the skin, improving the aqueous solubility, reducing irritancy over the skin and reducing higher penetrability through the skin. Higher penetrability of the drugs is controlled by controlling the release of the drugs over the penetration sites. Some of the nanocarriers provide good absorption in the skin cells. Better control over release of the drugs and improving absorption through the skin can overcome the problems like volatility and irritation over the skin (36, 37). In order to improve the topical deliverability, these nanocarriers are converted in to the semisolid dosage forms like hydrogels and creams (38).

1.7. Nanoemulgels

Most of the drugs under investigation or investigated are poorly soluble in water and considered lipophilic. This property led to reduced penetration through the biological membrane, low bioavailability, and non-uniform pharmacokinetic behaviour. In the recent few decaded, a nanocarrier based hydrogel approaches have gained much attention for delivering those types of drugs. Most specifically, the lipid-based nanocarrier have been used most extensively. Nanoemulgel has been emerged as a potential area of investigation as far as topical drug delivery is concerned. It demonstrates most tremendous advantages for the active moieties with poor biopharmaceutical attributes compared to conventional formulation. It is an example of semi-solid dosage forms consisting of oil/water nanoemulsion (NE) incorporated into the gel matrix to improve the therapeutic outcomes and pharmacokinetic profile of the lipophilic drug (39).

Nanoemulgels, which are a combination of emulsion based nanocarriers and hydrogels, offer several advantages in various applications. Here are some of the potential advantages:

1. Enhanced Stability: Nanoemulgels combine the stability of nanoemulsions with the viscosity and stability of hydrogels. This dual stability can help prevent phase separation and improve the overall stability of the formulation.
2. Improved Drug Delivery: Nanoemulgels are commonly used in pharmaceutical and biomedical applications for drug delivery. The nano-sized droplets in the emulsion can enhance the bioavailability of poorly soluble drugs, while the gel component allows for sustained release and prolonged therapeutic effects.

3. Increased Penetration and Absorption: The small droplet size in nanoemulsions can improve the penetration and absorption of active ingredients, making nanoemulgels effective for topical applications, such as in dermatology or transdermal drug delivery.
4. Targeted Delivery: Nanoemulgels can be designed to target specific tissues or cells, allowing for targeted drug delivery. This is particularly advantageous in the field of medicine, where targeted therapies can minimize side effects and improve treatment efficacy.
5. Versatility: Nanoemulgels can encapsulate a variety of substances, including hydrophobic and hydrophilic drugs, making them versatile carriers for a wide range of therapeutic agents.
6. Tunable Rheological Properties: The hydrogel component provides tunable rheological properties, allowing for easy application and adherence to different surfaces. This is particularly important in formulations designed for topical use.
7. Improved Solubilization: Nanoemulsions within the gel matrix can solubilize lipophilic compounds, improving their bioavailability and therapeutic effectiveness.
8. Extended Release: The gel component of nanoemulgels can control the release of the encapsulated substances, providing a sustained and prolonged release profile. This is advantageous for developing controlled-release formulations.
9. Enhanced Bioactivity: Nanoemulgels can protect bioactive compounds from degradation, preserving their bioactivity and enhancing their therapeutic effects.
10. *Cosmetic and Personal Care Applications*: Nanoemulgels are also used in the cosmetic and personal care industry for the delivery of active ingredients, such as vitamins and antioxidants. The small droplet size allows for better skin penetration, and the gel matrix provides a pleasant texture for application.

While nanoemulgels offer these advantages, it's essential to consider specific application requirements and potential challenges, such as formulation complexity and scale-up issues, in the development and commercialization of these systems.

2. Literature review

Psoriasis is considered as non-curable disease however its treatment mainly depends on the reduction of symptoms and modification of its progression. An array of topicals and systemic options are available in the market. However, progressive advancement in the investigations of effective treatment option for psoriasis is still in process. In 2005, Gottlieb et al. decided to study TNF/lymphotoxin blocking effect of etanercept in ten psoriasis patients treated for 6 months. This was because the less known mechanisms of action of Anti-TNF drugs in lesional tissues. Etanercept showed rapid alleviation in the level of IL-1β, IL-8 and other inflammatory genes, slow reduction in myeloid cells (CD11c+ cells) infiltration and T lymphocytes and the restoration of epidermal hyperplasia and parakeratosis (40).

New era of investigating the psoriasis exacerbation and its treatment started when Lowes MA et al. (2008) elaborated the importance of 17^{th} type helper T cells (Th-17) at inflammatory site and autoimmunity which is now getting widespread appreciation. They reported the presence of Th-17 cells in psoriatic lesions (dermis) and peripheral blood. Alleviation in the level of IL-17 mRNA was observed after administering cyclosporine systematically. Level of both IL-22 and IL-17 got reduced along with the reduction in keratin proliferation. Th-17 cells identified as the distinct population i.e. different from Th-1 and Th-2 type cells. They also identified that psoriasis is the result of responses of both Th-1 and Th-17 cells out of which Th-17 is the prime regulator and cause of the major psoriatic lesions. Therefore, they recommended that Th-17 cells should be the centre of attention for most of the drug discovery scientist and should be the most appropriate therapeutic target for various anti-psoriatic drugs (41).

Based on various targets, several anti-psoriatic drugs have been explored. The drugs which are available in the market reduce the symptoms associated with psoriasis. Based on route of administration, treatment options are classified in the following types viz. biologicals, systemics, phototherapies, oral treatments and topicals. Topical treatments are the first line of treatment in which topical corticosteroid is one of the most prescribed options along with methotraxate and cyclosporine. While, most of the prescribed drugs today are associated with serious side effects and cause inconvenience to the patients in long-term use, invention of new alternative treatments for psoriasis causing fewer side effects are in need.

Several plant-based drugs can meet these requirements. Their acceptance among patients is high due to their cost effectiveness, safety, and broad structural diversity.

As reported by Das et al. in 2017, significant evidences are available favouring the use of TCS in atopic eczema, localized vitiligo, psoriasis, chronic hand eczema and localized bullous pemphigoid. Not only dermatologists but quacks, general physicians, paediatricians, gynaecologists, and specialists of innumerable disciplines also prescribe steroids rampantly. Despite these facts, in long term use steroids are less preferred against psoriasis and increasingly being accused by doctors and patients. Topical steroid addiction and red burning skin syndrome are legitimate clinical entities which are well recognized these days. Sometimes, these terms are used synonymously. As a result, the problem of steroid phobia is being increasingly recognized by physicians worldwide which, sometimes associated with simple fear due to ignorance (42).

Due to the high degree of acceptability and potential to meet the above requirements, plant-based drugs are preferred over other conventional therapeutics. If the solution lies within the traditional or alternative system of medicine, it could be the best option to overcome the above problems up to the considerable extent. As per the available literatures, plant-based leads have been studied well against systemic as well as topical inflammations and some of the known examples are summarized here.

In 2009, Wang et al. discovered an alternative medication targeting NF-kB with a natural triterpenoid that was able to alleviate skin lesions of psoriasis in mice model. Acetyl-11-keto-beta-boswellic acid worked as I-κβ kinase inhibitor which reduced NF-kβ associated gene expression and the level of over produced cytokines. Therefore, targeting NF-kβ was proposed as useful target to treat psoriasis (19). Topical curcumin attenuated the IMQ Induced psoriasis and alleviated the level of IL-1β and IL-6 as reported by Sun et al. in 2013 (20).

In 2016, Lauro et al. reported the role of bergamot polyphenolic fraction in the attenuation of oxidative stress in spinal cord when studied in morphine induced tolerance and hyperalgesia model of mice. This was observed due to the alleviation of superoxide and malondialdehyde production, prevention of GS nitration and re-establishment of glutamate levels. Hence, this plant-based antioxidant was concluded as a vital option to restore opioids analgesic efficacy (21). Previously in 2013, the antitumor potential of bergamot essential oil

(BEO) has also been reported, when this oil was delivered in the form of liposomes on human neuroblastoma cells. This investigation was carried out by Celia et al. to overcome the problems associated with aqueous solubility and limited efficacy of this oil components which got improved after encapsulating oil in to liposomal systems (22). Wei et al. evaluated antioxidant/lipoxygenase inhibitory activity of bergamot oil and 24 other essential oils in 2010, where bergamot oil exhibited very significant anti-oxidant activity along with thyme oil in all the tested oils (23).

Eucalyptol (1,8-cineole), which is the main constituent of eucalyptus oil and known for anti-inflammatory activity, interacts with the transient receptor potential cation channels of various targets. Since it was unclear that, which of the targets mediate its anti-inflammatory effects, Caceres et al. 2017 carried out an investigation citing TRPM8 channels as the essential target for the anti-inflammatory effects of eucalyptol in mice. Human TRPM8 channels found more sensitive to eucalyptol than rodent TRPM8 channels explaining the higher potency of eucalyptol in humans. As metabolites of eucalyptol may also contribute to its anti-inflammatory effect, development of more potent and selective TRPM8 agonists may yield novel anti-inflammatory agents (24).

Anti-inflammatory and analgesic activity of *Cymbopogon citrates* and *Eucalyptus citriodora* oils were reported by Gbenou et al. in 2013. The main component of E. citriodora oil was observed to be citronellal (83.50 %) that showed dose dependent alleviation in oedema, strong analgesic and antipyretic effects as equivalent as produced by 50 mg/kg of acetylsalicylate. Aldehydes which are mainly present in these oils were supposed to act against oxidative stress and inflammation (25).

Juergens et al. 2003 reported the activity of eucalyptol against bronchial asthma in a double-blind placebo-controlled clinical trial. It suppressed arachidonic acid metabolic pathway and cytokine expression. This was the first report highlighted the potential of a monoterpene eucalyptol against asthmatic inflammation and provided the information about rational use of eucalyptol as mucolytic agent (43).

Subsequently, essential oil of *Zhumeria majdae* was reported to be anti-inflammatory in 2015 by Miraghazadeh et al. as this oil was observed as the rich source of linalool. In

results, this essential oil was observed as anti-inflammatory and anti-nociceptive agent. Hence it can be used in the management of pain and inflammation (44).

Anti-inflammatory effect of linalyl acetate has also been reported by Peana et al. in 2002. Results suggest that, linalyl acetate is less efficacious and its effect is prolonged as compared to its alcohol counterpart i.e. linalool on local edema when administered in equimolar concentration. They have observed linalyl acetate with some pro-drug like characteristics. They have hypothesized that, most of the anti-inflammatory properties an essential oil could be due to the presence of linalool and its acetate when both of them are present in significant concentration (45). Further, linalyl acetate (extracted from *Citrus aurantium* L. blossoms essential oil) was reported as an analgesic moiety. The major constituents present in this oil are linalool, linalyl acetate, nerolidol, E, E-farnesol, α-terpineol and limonene as reported elsewhere (46).

The anti-psoriatic potential of Betulin, that is the major component of *Betula utilis,* has been established specifically following Glut-1 receptor-based mechanism and validated using imiquimod (IMQ 5%) induced psoriasis-like skin inflammation model. Though it was found very effective against psoriasis like skin inflammation (27), betulin and its chemical derivatives, metabolites in their native form are considered poorly water soluble with very high logP value i.e., 6.17 limiting its direct use by oral route of administration (47). Since its logP value is very high, it would be great candidate for its topical application also as it would get retained in the lipidic environment of the skin and would not be permeated into the blood stream.

Despite LogP value, a molecule requires sufficient water solubility to overcome the physiological barrier of the skin, i.e., stratum cornium to get available inside the skin layers. While its solubility in water is very less. Therefore, there is need to modify its solubility and penetration ability to ensure better availability to reach the major payload inside the skin layers and ensure high effectiveness against psoriasis. It is non-toxic and classified as class VI toxic substance. This birch triterpene has been explored as an antiallergic, antiviral, antimicrobial, antitumor and hepatoprotective agent. It is safe to use on skin and in biological system (48).

As far as the dosage form designing of botulin for topical application is concerned, transforming nanocarrier systems in hydrogel system has been emerged as a novel and most useful area of research with tremendous industrial application and scalability (38). Nuchuchua et al. in 2009 reported that nanoemulsion avail drug to the site of topical administration for longer period ensure improved efficacy of the drug (49, 50). Santos et al. reported nanoemulsion as one of the most prospective candidates among various nanocarrier-based approaches due to its biocompatibility, biodegradability, and higher drug loading of hydrophilic or hydrophobic moieties (51) with industrial scalability. Alves et al. 2007 prepared hydro gel of nanoemulsion to make nanoemulsion topically deliverable, offer stationary compartment to the nanoemulsion droplets and keep nanoemulsion in intact form over the skin until drug absorption (52, 53). Cal et al. 2006 showed the effect of type of vehicles on *in-vitro* skin absorption and elimination kinetics of terpenes. Terpenes do not need to penetrate into viable skin tissue rapidly as they are widely reported as penetration enhancers in the topical dermal preparations, cosmetics and toiletries products. Nanogel offers higher skin absorption as compared to other formulation. In a study, total content of linalool and terpinen-4-ol observed after 4 h was 385 and 705 microg/cm^2 respectively. After 1 h of the elimination process about 10-20% drop of the total content of both terpenes in the skin was noted for all formulations. Order of vehicle capability to increase skin absorption of both terpenes was found in the order of: emulsion < oily solution < hydrogel (54).

3. Research envisaged

Topicals are considered the first line treatment option against mild to moderate psoriasis like conditions. The herbal bioactive based safe and effective topical formulation can be the best approach compared to the topical corticosteroids such as mometasone or dexamethasone that are current being prescribed in combination with methotraxate and cyclosporine. Many of these steroids are found to be associated with serious side effects, inconvenience, cutaneous atrophy, organ toxicity (hepatotoxicity, nephrotoxicity, teratogenicity), carcinogenicity and broadband immunosuppression, which are limiting their long-term use. However, their short-term use lead to the remission of the disease after finishing the treatment. Surveys carried out on psoriatic patients show high degree of disappointment due to the hopelessness associated with the available products in the market. Therefore, the invention of new alternative treatments for psoriasis with fewer side effects were desirable. Several plant-based drugs can meet these requirements and have been observed as promising agents for psoriasis treatment. Their acceptance among patients is very high as they are supposed to be safer than that of other conventional therapeutics. Broad structural diversity and multidirectional mechanisms of action make them a cost effective and efficient treatment option against psoriasis.

The anti-psoriatic potential of Betulin, that is the major component of *Betula utilis,* has been well established following Glut-1 receptor-based mechanism. Though it was found very effective against psoriasis like skin inflammation (27), betulin and its chemical derivatives, metabolites in their native form are considered poorly water soluble with very high logP value i.e., 6.17 limiting its direct use by oral route of administration (47). Since its logP value is very high, it would be great candidate for its topical application also as it would get retained in the lipidic environment of the skin and would not be permeated into the blood stream. Despite LogP value, sufficient water solubility is required to overcome the physiological barrier of the skin, i.e., stratum cornium to get available inside the skin layers. While its solubility in water is very less. Therefore, there is need to modify its solubility and penetration ability to ensure better availability to reach the major payload inside the skin layers.

Here we hypothesized that, a nano-hydro-colloidal systems-based hydrogel of betulin could enhance its distribution into the aqueous systems, increase skin penetration, ensure high skin availability and enhance therapeutic efficacy against psoriasis like inflammation.

Transforming nanocarrier systems in the form of gel is supposed to increase the viscosity, on account of which the retention time of the actives can be increased over the skin.

4. Aim and objectives of the study

Based on the above hypothesis, the objectives of the study are to develop and evaluate nanocarriers-based topical dosage forms of an anti-psoriatic bioactive betulin.

Our main objectives are

1. To develop and evaluate stable and scalable nanocarriers-based topical dosage forms of betulin
2. To enhance aqueous distribution and penetration of betulin for ensuring high skin availability and extended retention
3. To ensure safe and improved localized anti-psoriatic effect of betulin

5. Plan of work

A. Literature review

B. Procurement of the bioactive and ingredients

C. Pre-formulation Studies of betulin

1. ***Characteristics of the bioactive:*** Physical properties like the state of the molecules, colour and odour were observed visually.
2. ***Purity check and identification*** using HPLC and LCMS (55)
3. ***Determination of absorption maxima:*** UV-Vis spectroscopy
4. ***Preparation of standard curve in a suitable solvent***
5. ***Solubility:*** Saturation solubility studies
6. **LogP value** by shake flask method

D. Formulation and development

A. ***Development of nanoemulsion and lipid nanocarriers of betulin***

a) *Screening of ingredients for making nanoemulsion and lipid nanocarriers*

b) *Thermodynamic stability studies* for assessing phase separation, creaming or cracking

i. Heating-cooling cycle (4 °C and 45 °C)
ii. Freeze-thaw cycles (−21 °C and +25 °C)
iii. Infinite dilution

c) *Optimization of the experimental conditions using quality by design approach*

i. Defining the quality target product profile
ii. Identifying critical quality attributes by assessing risk with the help of Minitab 18 software and Ishikawa fish-bone diagram
iii. Experimental design using central composite design (56)

B. ***Preparation of the optimized nanoemulsion and lipid carrier dispersion***

C. ***Dialysis of the free drug***

D. ***Characterization of nanocarriers dispersion***

i. Surface morphology using SEM/TEM
ii. pH determination
iii. Viscosity
iv. Micrometric properties using particle size analyzer
v. Surface charge using zeta potential analyzer

vi. Drug entrapment efficiency and drug loading (57).

E. ***Preparation of nanoemulgels***

F. ***Characterization of the prepared gels***

G. Evaluation of the developed gels

i. *Viscosity and rheological behaviours*

ii. *Texture analysis*

iii. *Intactness of drug in the developed gel using analytical tool*

iv. *In vitro release study*

v. *Ex vivo permeation study*

vi. *Ex vivo skin deposition studies*

vii. *Ex vivo skin distribution study by confocal laser scanning laser microscope*

viii. *In vivo investigations*

1. *Experimental design of the anti-psoriatic activity and induction of the disease*

2. *Assessment of anti-psoriatic activity*

3. Psoriasis Area and Severity Index Scoring

4. Investigation of spleen morphology

5. Biochemical estimation

6. Macroscopic and behavioral investigations

7. Safety evaluation by assessing primary irritation index as per OECD guideline 404 (36).

ix. *In vitro cytotoxicity* using MTT assay (58)

E. Stability study: As per ICH guidelines of the formulation being developed for climate zone III and IV.

F. Statistical analysis Data will be presented as mean ± standard error means (SEM). Data for treatment groups will be compared either using t test or one-way analysis of variance (ANOVA). All statistical analyses will be performed using GraphPad Prism, Version 5.01 (GraphPad software. Inc., USA). The statistical significance of differences will be accepted at the level of $p<0.05$.

G. Data compilation and analysis

H. Publication and securing rights of intellectual property

I. **Thesis submission**

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